United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Microbiological Data Program

SOP No: MDP-MTH-02		Page 1 of 11
Title: S*almonella* VIDAS® Method		
Revision: 02	Replaces: 08/15/03	Effective: 01/01/04

1. Purpose:

1.1. To provide standard procedures for screening Microbiological Data Program (MDP) fruit and vegetable samples for *Salmonella* using the VIDAS® method, based on an antigen-antibody reaction detected by enzyme-linked fluorescent immunoassay (ELFA).

1.2. This SOP shall be used as a backup method in the event of problems encountered in the operations of BAX-PCR.

2. Scope:

2.1. This standard operating procedure (SOP) shall be followed by all laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities that may impact the program.

3. Principle:

3.1. The VIDAS® (Vitek Immuno Diagnostic Assay System) SLM principle is described in the VIDAS® SLM package insert and the website http://industry.biomerieux-usa.com/

4. Outline of Procedures:

4.8. Quality Assurance 6.8

5. References:

5.1. Andrews WH, and Hammack TS. *Salmonella* (Chapter 5). BAM online. April 2003. http://www.cfsan.fda.gov/~ebam/bam-5html

5.2. VIDAS. bioMérieux. User Guide and Package Insert

5.3. Curiale, M.S., Gangar, V. and Gravens, C. 1997. VIDAS enzyme-linked immunofluorescent assay for detection of *Salmonella* in food: collaborative study. Journal of AOAC International, Volume 80, No.3, pp. 491-504

5.4. USDA, AMS, MDP-LABOP-07 DRAFT, Maintenance of *Salmonella* and *E. coli* Positive Control Cultures with GFP Plasmid: DRAFT, Revision: Draft 02-04/03.

5.5. Maijala R, Johansson T, and Hirn J. 1992. Growth of Salmonella and competing flora in five commercial RV media. International Journal of Food Microbiology. Vol.17, pp.1-8.

6. Specific Procedures: (Follow manufacturer's instructions for methodology, instrument set-up, precautions and warnings)

6.1. Media and Reagents

6.1.1. VIDAS® *Salmonella* (SLM) assay kit (available from bioMérieux, Inc., 595 Anglum Road, Hazelwood, MO 63042-2320)

6.1.2. Lactose broth

6.1.3. Rappaport-Vassiliadis (RV) Broth - 16 x 150 mm sterile test tubes containing 10 mL aliquots. (NOTE: RV broth by Oxoid has been shown to provide better enrichment of *Salmonella* compared to RV produced by other manufacturers. Therefore, Oxoid RV broth is recommended.)

6.1.4. Tetrathionate (TT) broth (with iodine and brilliant green) - 16 x 150 mm sterile test tubes containing 10 mL aliquots (day medium used add 20 mL iodine solution per 1 liter basal broth and 10 mL brilliant green solution per 1 liter basal broth)

6.1.5. Iodine solution for basal TT broth

6.1.6. 0.1 % (w/v) Brilliant Green solution for basal TT broth

6.1.7. M-broth - 16 x 150 mm test tubes containing 10 mL aliquots

6.1.8. *Salmonella enterica* serovar Poona/ pKT-kan, containing a plasmid that carries a gene coding for green fluorescent protein (GFP), as a positive culture control

6.1.9. *Escherichia coli/* pKT-kan containing a plasmid that carries a gene coding for GFP, as a negative control

6.1.10. *Enterobacter aerogenes*, as a negative culture control

6.1.11. Buffered peptone water plus 0.1% Tween 80 (MDP-LABOP-02)

6.1.12. 1 N Sodium hydroxide (NaOH) solution

6.1.13. 1 N Hydrochloric (HCl) acid solution

6.2. Apparatus

6.2.1. Pipette or micropipet with disposable tip calibrated to dispense 0.5 mL (500 µL)

6.2.2. Boiling waterbaths capable of attaining and maintaining a temperature of 95-100°C .

6.2.3. VIDAS® automated immunoassay system from bioMérieux, Inc.

6.2.4. Balance, top loading, minimum 1,000 g capacity, sensitive to 0.1 g

6.2.5. Incubators - constant temperature 35±1°C and 42°±0.5°C

6.2.6. Waterbath - thermostatically controlled and constant temperature 42±0.2°C

6.2.7. Waterbath - thermostatically controlled and constant temperature 35±0.2°C

6.2.8. Sterile culture type test tubes

6.2.9. Pipettes, sterile, disposable, 10 mL with 0.1mL graduations

6.2.10. Pipettes, sterile, disposable, 2.0 mL with 0.1mL and 0.5mL graduations. May use adjustable volume macropipetter with disposable tips if desired.

6.2.11. A pipette aide is required for transfer pipettes.

6.2.12. Inoculation loops and needles. May use plastic, sterile disposable 3 mm i.d. loops (10 µL) and plastic, sterile disposable needles if desired.

6.2.13. Vortex mixer

6.2.14. Thermometers, one immersion type, or digital probe with 0.1^{o} subdivisions and one thermometer with a range that includes 35^{o}C. Both thermometers calibrated to a standard thermometer that is certified by a National Institute of Standards and Technology (NIST) thermometer.

6.3. Preparation of Test Broths

6.3.1. Pre-enrichment

6.3.1.1. Pre-warm lactose broth to room temperature.

6.3.1.2. Aseptically add 25±1 g or transfer 25±1 mL of the sample eluate (See SOP MDP-LABOP-02) to 225±5 mL of sterile lactose broth in a suitable sterile container.

6.3.1.3. Incubate lactose broth for 18-24 hours at 35 ± 1°C.

6.3.2. Selective Enrichment

6.3.2.1. After incubation, swirl lactose broth to mix, and transfer 0.1 mL of the culture suspension into 10 mL of RV broth. Incubate 18-24 hours in a 42±0.5°C waterbath.

6.3.2.2. In addition, transfer 1mL of the culture suspension into 10 mL of TT broth. Incubate 18-24 hours in a 42±0.5°C waterbath

6.3.3. Post-enrichment

6.3.3.1. After incubation, mix selective enrichment broths using a vortex mixer or by pipetting. Transfer 1 mL from the RV broth into 10 mL of M-broth. Transfer 1 mL of TT broth into another 10 mL of M-broth.

6.3.3.2. Incubate both M-broth samples for 6-8 hours at 42±0.5°C in an incubator or at 42± 0.2°C in a waterbath. If the VIDAS assay has to be delayed, the M-broths can be stored for up to 48 hours at 2-8 °C.

6.3.3.3. After incubation, mix M-broth tubes using a vortex mixer or by pipetting. For each sample, combine 1 mL from each M-broth into a sterile test tube. Store remaining M-broths at 2-8°C and use for confirmation of positive results.

6.4. Enzyme Immunoassay Procedure

6.4.1. Heat the samples in M-broth combination (from section 6.4.3.4) by submerging the broth level for the test tubes in a boiling waterbath for 15 minutes to inactivate microorganisms.

6.4.2. Cool heated extracts to room temperature (20-25°C) prior to running enzyme immunoassay analysis.

6.4.3. Vortex the provided standard (S_1) and control samples (C_1 & C_2). Pipette 0.5 mL (500 µL) of each into the center of the sample well of the appropriately labeled SLM Reagent Strip. The standard (S_1) should be run in duplicate.

6.4.4. Vortex the boiled samples (as prepared in 6.5.1.). Pipette 0.5 mL (500 µL) of each sample into the center of the sample well of a SLM Reagent Strip.

6.4.5. Set up assay and instrument as per manufacturer's instructions.

6.4.6. Analyze samples.

6.4.7. Final results will be printed as "negative" or "positive". A "negative" result is reported as *Salmonella* negative and the test is concluded. A "positive" result must be confirmed.

6.5. Confirmation of Assay Positive Results

6.5.1. Positive ELFA reading indicates S*almonella* sp. may be present. However, VIDAS® SLM assay positive results are considered presumptive and must be confirmed from the

stored 6-8-hour cultures in M-broth. Confirmations are performed according to S*almonella* culture methods in the MDP-MTH-03.

6.6. Quality Assurance

6.6.1. Procedure for preparing organisms for use as positive and negative controls

6.6.1.1. On the day prior to the testing of a group of produce samples, inoculate a fresh broth of the following organisms control strains and incubate at 35°C overnight:

6.6.1.1.1. *Salmonella enterica* serovar Poona/pKT–kan

6.6.1.1.2. *Escherichia coli* pKT–kan

6.6.1.1.3. Enterobacter aerogenes

6.6.1.2. On the day of testing, small volumes (<100 mL.) of lactose broth are each inoculated with the organisms and incubated 4-5 hours at 35°C to ~0.5 McFarland turbidity (faintly turbid visually). This suspension will be used when setting up the quality control during testing.

6.6.2. The following controls are included and used in the MDP Salmonella setup.

6.6.2.1. Negative media control: 25 mL buffered peptone water + 0.1% Tween 80 (See SOP MDP-LABOP-02) to 225 mL sterile lactose broth.

6.6.2.2. Negative culture control: 1 mL *E. aerogenes* suspension in 225 mL sterile lactose broth.

6.6.2.3. Positive *Salmonella* pure culture control: 1mL *S. enterica* serovar Poona culture suspension in 225 mL sterile lactose broth.

6.6.2.4. Positive produce culture control: A single produce sample chosen at random after eluate is inoculated into test cultures has the following additions of the culture suspensions from 6.8.1.2. combined: 1 mL *E. coli*, and 1 mL *S. enterica* serovar Poona. Gently mix produce by hand – do not use shaker; 1 mL of the produce control eluate is inoculated into 225 mL of sterile lactose broth.

6.6.3. Use the controls prepared in step 6.8.2 for each daily batch of samples and carry forward through the remaining test broth preparation steps, the SLM VIDAS® assay steps, and the culture confirmation steps if a confirmation is needed on a sample. (See SOP MDP-MTH-03). If the positive control fails to yield a satisfactory result, or there is any question about the performance of the testing because of the control results, refer to SOP MDP-QA-01.

6.6.4. The VIDAS® analysis should be performed immediately after the heat killed samples of the M-broth cultures have cooled. If this cannot be done, the non-boiled M-broths can be stored for up to 48 hours at 2-8 °C. If longer storage is required, freeze the aliquot to be used before heating.

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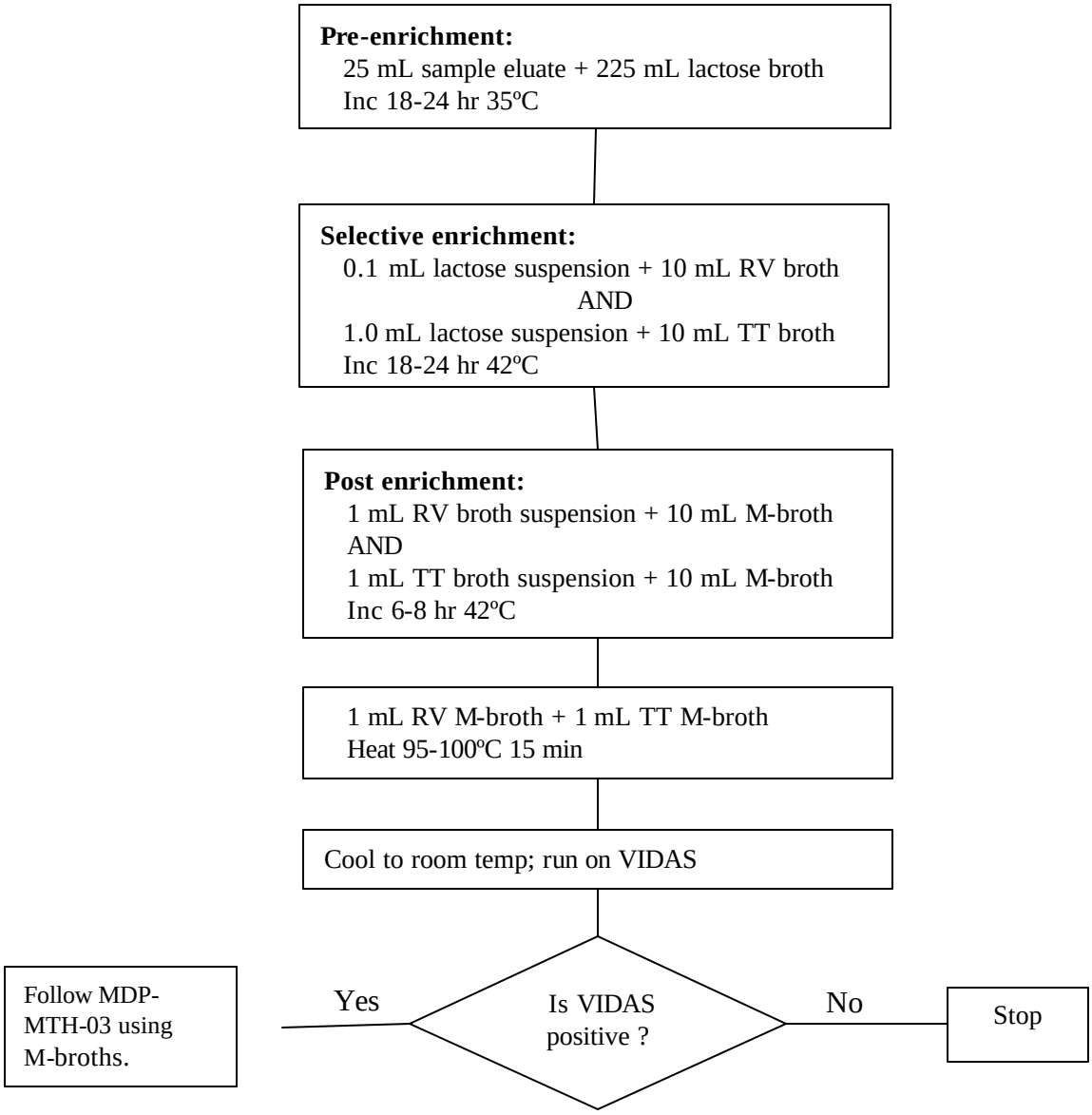
ELECTRONICALLY REPRODUCED SIGNATURES

Revision 02	Date	MPO

- Adjusted purpose to indicate VIDAS® as backup method in case of failure of BAX instrument methods
- Changed from Selenite Cystine Broth to Rappaport-Vassiliadis broth and recommended use of Oxoid RV Broth.
- Changed from Butterfield's Phosphate Buffer + 1% Tween to Buffered Peptone Water + 0.1% Tween
- Changed from 50 mL sample eluate + 450mL lactose broth to 25 mL sample eluate + 225 mL lactose broth (25g is a serving size)
- Removed the step for testing pH after 1 hr incubation of inoculated lactose broth
- Increased incubation of TT & RV broth to 18-24 hours at 42±0.5°C
- Decreased incubation of M-broth to 6-8 hours
- Changed amount of broth used for controls to 225 mL lactose broth
- Removed sections on operation of VIDAS instrument; laboratories are advised to refer to the manufacturer's instructions. Work instructions may be written by each laboratory for the operation of the VIDAS instrument.


Pre-enrichment:
25 mL sample eluate + 225 mL lactose broth
Inc 18-24 hr 35ºC
Selective enrichment:
0.1 mL lactose suspension + 10 mL RV broth
AND
1.0 mL lactose suspension + 10 mL TT broth
Inc 18-24 hr 42ºC
Post enrichment:
1 mL RV broth suspension + 10 mL M-broth
AND
1 mL TT broth suspension + 10 mL M-broth
Inc 6-8 hr 42ºC
1 mL RV M-broth + 1 mL TT M-broth
Heat 95-100ºC 15 min
Cool to room temp; run on VIDAS
Is VIDAS positive ?
Yes
No
Follow MDP-MTH-03 using M-broths.
Stop